REMARKS/ARGUMENTS

Claims 40-50 and 55 are pending in the above-identified application. Claims 40 has been amended. Support for this amendment is identified in the following remarks. No new matter is added by these amendments.

Rejections under 35 U.S.C. §112, first paragraph

Claims 40-50 and 55 remain rejected under 35 U.S.C. § 112, first paragraph, the Examiner alleging that the specification, while being enabling for the use of retaining glycosidases wherein the catalytically active carboxylic acid that is the nucleophile is mutated to form an oligosaccharide using a specific glycosyl fluoride as a donor and a specific acceptor, does not reasonably provide enablement for a method for synthesizing any oligosaccharide using any glycosidase and any donor and acceptor molecules. The Examiner contends that it is "unpredictable whether any glycosidase when mutated at any one of two catalytically active carboxylic amino acids will catalyze coupling of any glycosyl donor and any glycosyl acceptor having opposite stereochemical configurations by either inverting or retaining mechanism."

Applicants must again respectfully traverse this rejection. First, contrary to the opinion of the Examiner Applicants have not argued that the present invention is directed to a method for synthesizing any oligosaccharide using any random mutated glycosidase and any randomly selected donor and acceptor molecule. The present invention instead, and as previously stated, broadly teaches the mutation of a glycosidase, both retaining and inverting, at one of two catalytically active amino acids in the active site of the enzyme to result in an enzyme that has lost the ability to hydrolyze oligosaccharide products, but that retain the ability to couple corresponding donor and acceptor molecules. Generally, the substrate specificity of the wild-type enzyme is retained. Support for this contention is found in the specification as filed and in the various references that have been provided by Applicants during prosecution. Further, Applicants provide attached hereto a further Declaration of Dr. Stephen G. Withers ("the Withers Declaration"), a co-inventor of the pending claims, to provide a further description of the

scientific principles that underlie this concept. (Paragraphs 3 through 5 of the Withers Declaration). Therefore, the claimed invention is not directed to a method for synthesizing any oligosaccharide using any random mutated glycosidase and any random donor and random acceptor molecule. Although Applicants traverse this rejection, but in order to further expedite prosecution of the present application, Applicants have amended claim 40 to recite "carboxylic acid side chains within the active site of the wild-type enzyme that corresponds to the donor molecule and the acceptor molecule to be coupled". This amendment is not believed to limit the claim in any manner and is made only to clarify the subject matter of the claimed invention.

The selection of a desired final product or the selection of a particular mutated glycosidase and the specification provide the skilled artisan with sufficient guidance to select the appropriate corresponding mutated glycosidase or donor molecule and acceptor molecule as the terms are used in the pending claims. As above, the mutated glycosidase generally retains the substrate specificity of the wild-type enzyme. The enzymatic reaction catalyzed by a mutated glycosidase (a glycosynthase) of the present invention is well known to be a nucleophilic substitution reaction. This type of reaction is well known to the skilled artisan and is considered predictable in the art. Therefore, the skilled artisan reading the specification as of the effective filing date of the present application would reasonably interpret the term "modified glycosyl donor" recited in the claims to mean a glycoside molecule modified by a group that functions as a "good leaving group" (*i.e.*, a group that makes the donor more reactive). The skilled artisan is also provided with the guidance that the leaving group must be "reasonably small" in order to fit within the active site of the particular enzyme under consideration. (See specification at, *e.g.*, page 12, line 3-11 and Withers Declaration paragraph 6).

As further provided in the Wither Declaration (paragraph 7), groups that function as good leaving groups in a nucleophilic substitution reaction were well known in the art as of the effective filing date of the present application. As an example, the standard organic chemistry text entitled "Mechanism and Theory in Organic Chemistry", 3rd Edition, Lowry and Richardson eds. (1987), among others, provide lists of various groups that could be used in these reactions. Among the listed leaving groups are, for example, fluoride, chloride, formate, acetate,

propionates, pivaloates, and substituted phenols. (See Withers Declaration paragraph 7). It is further known in the art that azide and formate are smaller than or similar in size to the leaving groups listed as examples in the specification.

The Examiner has determined that the Declaration of Dr. Stephen G. Withers filed November 24, 2003 is insufficient to overcome the rejection under 35 U.S.C. § 112, first paragraph, because the Examiner believes that the examples of leaving groups used in the experiments, azide and formate, are not listed in the specification and the experiments presumably were carried out after the filing date of the instant invention. Prior to responding to the determination of the Examiner regarding the Withers November 24, 2003 declaration, it should be noted that the Examiner has not yet presented a reasoned basis for why the use of a specific donor species in the present application and in post filing examples does not establish enablement for the pending claims. A reasoned basis for the rejection is required prior to shifting the burden for establishing enablement to the applicants.

As to the determination of the Examiner that the Withers November 23, 2003 declaration is insufficient to overcome the enablement rejection, the Examiner is respectfully requested to review paragraphs 6 and 7 of the Withers declaration attached hereto, the specification and all prior submitted declarations and attachments. As above, good leaving groups for nucleophilic displacement reactions are well known in the art. Several good leaving groups are provided in the specification as filed (page 12, lines 3-11) and additional leaving groups were known at the effective filing date of the present invention (*e.g.*, Mechanism and Theory of Organic Chemistry). Further, azide and formate were known to be substrates of a mutant glycosidase enzyme (*Agrobacterium faecalis* β-glucosidase) in a rescue reaction (Wang *et al.*, *J. m. Chem. Soc.* 116:11594-11595 (1994). Wang *et al.*, also provide certain information relating to the average distance between each pair of active site carboxylate oxygen atoms for a number of glycosidase enzymes (Withers declaration, paragraph 7). The skilled artisan armed with the specification as filed and information available in the art prior to the effective filing date of the present invention would have had sufficient guidance to select a number of leaving groups of the proper size to modify a selected donor molecule. No undue experimentation would be

required of the skilled artisan in selecting the donor or the acceptor for a corresponding mutant glycosidase and a reasonable expectation of success for a selected modification of a particular donor and acceptor pair.

In order to further expedite prosecution additional data that has been recently acquired is provided in the Withers Declaration attached hereto. The experiments described in paragraphs 8 through 11 of the Withers Declaration demonstrate the use of α-glucosyl azide donor as an altermate donor to α-glycosyl fluoride with a mutant β-glycosidase. This experiment is similar to the November 23, 2003 Withers Declaration in that azide is used as the small leaving group and that azide is not listed in the specification as an example of a leaving group. In this experiment the method is more similar to that described in the specification. The generation of the modified donor molecule is carried out separately from the transglycosylation reaction as described in the specification. In particular, azide was reacted with per-O-acetylated β-glucosyl chloride to give the protected α-glucosyl azide. The protected α-glucosyl azide was then deprotected with sodium methoxide in methanol. This purified and characterized modified donor molecule was used in the enzymatic reaction with the corresponding mutated glycosidase. (See paragraph 10 of the Withers Declaration attached hereto). In the glycosynthase reactions, the modified α-glucosyl azide donor was transglycosylated onto the glycoside acceptor (*p*NP β-D-glucoside). The α-glucosyl azide donor was incubated in the presence of *p*NP β-D-glucoside with the corresponding nucleophile mutant *Agrobacterium* E358G β-glucosidase (AbgE358G). The data demonstrate that, using the azide modified α-glucosyl donor and an acceptor molecule that correspond to the mutant glycosidase as described in the specification a transglycosylation reaction occurred is indicate by the appearance of new synthetic products. Therefore, based on the guidance provided in the specification as filed, the knowledge of the skilled artisan as of the effective filing date of the present application a method for the preparation of an oligosaccharide has been fully enabled as if the effective filing date of the application. The method comprises enzymatically coupling a modified glycosyl donor molecule and a glycoside acceptor molecule in a reaction mixture using a mutant form of glycosidase enzyme to form the oligosaccharide. The mutant enzyme is selected from among glycosidase enzymes having two catalytically active

amino acids with carboxylic acid side chains within the active site of the wild-type enzyme that corresponds to the donor molecule and the acceptor molecule to be coupled. The mutant enzyme is mutated to replace one of the catalytically active amino acids having a carboxylic acid side chain with a different amino acid of comparable or smaller size, the different amino acid having a non-carboxylic acid side chain characterized in that, the modified glycosyl donor molecule having a β configuration and the glycoside acceptor molecule have an α configuration, or vice versa.

Further, Applicants have taught that a glycosidase enzyme can be mutated at one of two catalytically active carboxylic acid side chain containing amino acids in the active site of the enzyme to produce an enzyme that no longer hydrolyzes an oligonucleotide substrate, but can transglycosylate a corresponding modified glycosyl donor and an acceptor glycoside. In the case of a retaining enzyme the normal nucleophilic amino acid within the active site is changed to a non-nucleophilic amino acid. While in the case of an inverting glycosidase, the mutant enzyme is one in which the amino acid which normally functions as a base is replaced by a non-ionizable amino acid. Applicants have also disclosed the catalytic amino acids that are replaced in a number of enzymes and have taught various methods for determining which amino acid is replaced for those enzymes that the catalytic amino acids may not have been identified. It should be noted, as discussed in the prior responses, that the catalytic amino acids of a number of glycosidases were known at the date of filing the earliest claimed priority application, and that since that time the catalytic amino acids of a large number of additional enzymes have been determined by one or more of the methods disclosed in the application. Still further, Applicants have provided a number of post filing examples of mutated glycosidase enzymes that function as disclosed in the present application. With the present response Applicants provide additional examples of the use of modified glycosyl donor species in addition to glycosyl fluoride donors. It is well settled that a working example is not required if the invention is otherwise disclosed in such a manner that one skilled in the art would be able to practice it without undue experimentation. (MPEP § 2164.02). In the present case, Applicants have provided the skilled artisan sufficient guidance that in combination with knowledge in the prior art there is a

reasonable expectation that one can carry out each of the steps of the claimed methods. As in prior office actions, the Examiner has again provided no reasoned basis to doubt the enablement of either inverting or retaining enzymes. Therefore, Applicants believe the present invention has been fully enabled for the full scope of the claims.

Applicants respectfully request the Examiner to reconsider and withdraw this rejection in light of the amendment to the claims the attached declaration and the above remarks.

Rejections under 35 U.S.C. §112, second paragraph

Claims 40-50 and 55 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner believes that the term "modified glycosyl donor" is indefinite and that the specification defines the term by non-limiting examples rendering the metes and bounces of the claims unascertainable.

Although Applicants do not believe the term "modified glycosyl donor" is indefinite in the context of the present invention, claim 40 has been amended to recite "wherein said mutant enzyme corresponds to the donor molecule and the acceptor molecule pair and said mutant enzyme". Support for this amendment can be found at, for example, page 7, lines 1-8, page 8, lines 17-25, and throughout the specification. It is a basic tenant of the present invention that the glycosyl donor molecule, the glycoside acceptor molecule and the mutant glycosidase enzyme are generally selected to correspond to one another. As set forth in the attached Withers Declaration, the mutant glycosidase generally retains the substrate specificity of the wild-type glycosidase. (Paragraph 3 and 4). Some difference in substrate specificity, either more limited or more expanded, would be expected depending on the size of the amino acid residue substituted for the catalytically active amino acid having the carboxylic acid side chain, but this variation would not be sufficient to render the term indefinite. Therefore, the skilled artisan would have sufficient guidance in the specification alone, and in combination with the available art at the effective filing date or the present application to ascertain the metes and bounds of the

various donor and acceptor molecules for any selected mutant glycosidase. Applicants therefore respectfully request the Examiner to reconsider and withdraw the present rejection in light of the above amendments and remarks and the attached Withers Declaration.

Double Patenting Rejections

Claims 40-50 and 55 remain rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,716,812. Claims 40-50 and 55 also remain rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1 and 2 of U>S. Patent No. 6,284,494. As set forth in Applicants prior responses, the need for a terminal disclaimer will be evaluated and submitted, if required, once an indication that the claims are otherwise in condition for allowance has been received.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 24 August 2004 By: Brian W. Poor

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